REMARKS:

This is a full and complete response to the Office action dated November 29, 2007.

REGARDING THE CLAIMS:

Claims 11-12, 14-18, 23-30 are pending in the application. Claim 11 has been amended with support found in the application on page 10, table 1. Claim 14-16 have been amended for clarification and to adjust dependency. New claims 23-26 have been added with support in former claims 4-5 and 7-8. Additionally, new claims 28 and 30 claims having support in previously canceled claims. Claim No new matter has been added.

IN RESPONSE TO THE OFFICE ACTION:

REJECTION UNDER 35 U.S.C. § 112, 1ST PARAGRAPH:

Claims 11-12 and 16-18 stand rejected under 35 USC §112, first paragraph, as containing subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the art that the inventor(s), at the time the application was filed, had possession of the invention. Applicants respectfully traverse this rejection.

In particular the Examiner argues that the specification describes a method of hydoxylating fatty acids described in Examples 2-4 using a specific cytochrome P450 monooxygenase obtained from Bacillus megaterium and zinc/Co(III)sepulchrate electron donor system, however, these examples are not enough and do not constitute a representative number of species to describe the whole genus of any or all variants, recombinants and mutants of any or all polypeptides having cytochrome P450 monooxygenase activity.

Applicants note that claim 11 has been amended to recite "cytochrome P450-containing mono-oxygenase (E.C. 1.14) of the family CYP4, CYP52, CYP102." As noted in the application on page 10, table 1, the CYP4, CYP52, CYP102 families are known to catalyze the terminal and/or subterminal oxidation of fatty acids. As noted in the application on page 12, P450 BM-3 is of the CYP102 family. Applicants respectfully

submit that in view of the recitation of these related families one of skill in the art would easily understand the inventors had possession of the invention. Therefore for this reason alone Applicants respectfully submit the above mentioned rejection be withdrawn.

Applicants also submit herewith as evidence a copy of a publication co-authored by one of the present inventors, Nazor et al, "*Laboratory evolution of P450 BM3 for mediated electron transfer yielding an activity-improved and reductase-independent variant*", Protein Engineering, Design & Selection, pgs 1-7, 2007 (hereinafter "**Nazor**"). In this publication, several experimental results were ogbtained for P450 BM3 mutants M1 and M2. The corresponding mutated amino acid position can be seen from table 1 in the reference, (1) for M1 positions 47 and 87 were substituted, (2) positions 47, 87, and 354 were mutated. As noted, M1 is a double mutant, and M2 is a triple mutant. Furthermore, experimental results for these mutants are shown in Fig 1 of the reference. As shown therein, each of said mutants M1 and M2 hydroxylate the substrate (12-pNCA) in the presence of different electron sources (NADPH or Zn/Co (III)sep). Therefore, based on the originally disclosed experimental results (tables 2, 4, 5, and 6 of the present application) and the additional experimental evidence shown in **Nazor**, Applicants respectfully submit that the operability of the claimed method is shown not only for the wild type BM3 enzyme, but also for a panel of single and multiple mutants.

Therefore for this additional reason also possession of the claimed invention is demonstrated. The claimed method recites the structure of the enzyme and additionally variations and mutants of the wild type are sufficiently described as also shown by the results of **Nazor**. Therefore, for this reason also Applicants respectfully request the above mentioned rejection be withdrawn.

Applicants also note that on page 6, line 36 of the present application DE-A-100 11 723 is incorporated by reference and corresponds to US application 10/031,695. In this '695 application BM3 mutants containing from 1 to 5 mutations are disclosed and described as having the ability to hydroxylate fatty acids of different chain length. Therefore, the '695 application additionally supports the recitation of the enzyme as in claim 11.

Furthermore, with respect to the reference of the electron donor system as recited in claim 11 "the source of electrons is a metal in powder form with a lower standard normal potential than the mediator" Applicants note that enzymatic activity (and the ability to transfer electrons between the donor system and the substrate) is not destroyed despite multiple mutations performed within the enzyme sutructure. Moreover, the **Nazor** reference illustrates that enzyme activity is observed in the presence of NADPH or Zn/Co (III)sep as electron sources. It should be noted that these are completely different electron sources. One of skill in the art would therefore expect that related electron donor systems based on metal powder as an electron source and a mediator which able to transfer electrons from the electron source to the enzyme will be operable as well. For this reason also, Applicants respectfully assert that one skilled in the art would understand that at the time the application was filed, Applicants had possession of the invention.

For the above reasons therefore, Applicants respectfully request the above mentioned rejection be withdrawn.

REJECTION UNDER 35 U.S.C. § 112, 1ST PARAGRAPH:

Claims 11-12 and 16-18 stand rejected under 35 USC §112, first paragraph, for lack of enablement. Applicants respectfully traverse this rejection.

The Examiner asserts that while the specification is enabling for a method of hydrxylating fatty acids as described in Examples 2-4 of the specification using a cytochrome P450 monooxygenase obtained from *Bacillus megaterium* and Zinc/Co(III) sepulchrate, the application does not reasonably provide enablement for such a method of hydroxylating fatty acids using any or all variants, mutants and recombinants of any or all cytochrome P450 monooxygenase and any or all electron donor systems comprising any or all electrode-bound source electrons.

Enablement under 35 USC §112 requires that the specification describe the invention in such terms that one skilled in the art can make and use the claimed invention. The test is whether a person skilled in the art can make and use the invention without undue experimentation. *See* MPEP §2164.01.

Appellants respectfully assert that in view of the state of the art as well as the examples and significant direction provided in the Application, one of ordinary skill in the art could make and use the claimed invention without undue experimentation.

Furthermore, Appellants respectfully re-assert the remarks made above with respect to amend claim 11 and claims depending therefrom. As claim 11 recites "cytochrome P450-containing mono-oxygenase (E.C. 1.14) of the families CYP4, CYP52, CYP102," Applicants respectfully assert that the Examples contained in the present application with respect to BM-3 *Bacillus megaterium* are more than sufficient to enable one of skill in the art to make and use the claimed invention.

As further evidence, Applicants provide the **Nazor** reference as noted above which shows successful hydroxalation of the substrate (12-pNCA) in the presence of different electron sources (NADPH or Zn/Co (III)sep). Thus the enzyme with multiple mutations is demonstrated hydroxylation in two completely different electron sources. Furthermore, as noted above, in the '695 application BM3 mutants containing from 1 to 5 mutations are disclosed and described as having the ability to hydroxylate fatty acids of different chain length. Therefore, Applicants respectfully submit that undue experimentation would not be required for one of skill in the art to make and use the claimed invention. Accordingly, Applicants respectfully request the above mentioned rejection bet withdrawn.

REJECTION UNDER 35 U.S.C. § 103:

Claims 11 – 12 and 16 – 18 stand rejected under 35 U.S.C §103(a) over Estabrook et al. ("**Estabrook**"), in view of Creaser et al. ("**Creaser**") and Conrad et al. (J. Am. Chem. Soc. 1989, 111, 3461-3463) entitled "Long-Range Electron Transfer in a Cytochrome c Derivative Containing a Covalently Attached Cobalt-Cage Complex" ("**Conrad**"). Applicants respectfully traverse this rejection and respectfully submit that no prima facie case of obviousness can be established as hereinafter described.

The Examiner asserts that **Estabrook** discloses a method for the enzymatic production of terminally or subterminally hydroxylated fatty acids comprising hydroxylating fatty acids in the presence of an electron donor system, a cytochrome P450 monooxygenase, oxygen, chloride ions and a hydrogen peroxide cleaving enzyme. The

Examiner contends that the difference between the reference of **Estabrook** and the claimed invention is that **Estabrook** does not teach a method of producing terminally or subterminally hydroxylated fatty acids using a Zn metal powder form.

The Examiner further asserts that **Creaser** discloses a Zn/Co(III)sepulchrate electron donor system, and teaches that Zn dust causes reduction of the Co(III)sepulchrate mediator within seconds.

In light of this, the Examiner concludes that it would have been obvious to one of ordinary skill in the art to use either Zn dust as originally taught by **Creaser** or Pt as taught by **Estabrook** in hydroxylating fatty acids using a metal/Co(III)sepulchrate electron donor system**.**

The Examiner further states that **Conrad** discloses general use of Zn/Co(III) sepulchrate in electron transfer reactions in biological systems and thus one of ordinary skill in the art would apply the **Estabrook** and **Creaser** to biological systems.

Applicants respectfully assert that there is not the slightest piece of guidance in **Creaser** to use Zn dust in a biochemical system. There is no experimental guidance, nor is there even hypothetical guidance in the cited reference. To the contrary, at the end of the reference, it is speculated to use in organic and inorganic chemistry to use as therapeutic agents – still without any experimental guidance. *See* **Creaser**, pg. 3182, right col., 2nd ¶. Potential biochemical applications are not at all mentioned.

With respect to **Conrad** Applicants respectfully submit that the reference is completely irrelevant for the subject matter of the present claims. Additionally, the subject matter is such that one of skill in the art would have no reason to combine with the disclosures of **Estabrook** and/or **Creaser**. As noted in the title of the portion referenced by the Examiner, the cobalt-cage complexes therein are covalently attached (to the protein to horse heart cytochrome c). Said cytochrome c protein contains a large heme group and the intromolecular electron transfer from Co(II) of the covalently attached cage to the Fe(III) atom of the heme group was investigated. Contrary to this, the present claims are based on the electron transfer from zinc powder (not covalently attached to the P450 enzyme, but being present in the reaction medium) and the mediator (for example Co(III)sp-also not covalently attached to the enzyme. Therefore, one of

skill in the art would have no reason to combine with **Estabrook** and/or **Creaser**, nor would one of skill have any expectation of success with respect to the disclosure of **Conrad**, and therefore the reference does nothing remedy the defect in disclosure of **Estabrook** or **Creaser**.

Appellants also respectfully note that there is not the slightest suggestion or piece of guidance in **Estabrook** to replace the highly sophisticated electrode system by a metal powder. Furthermore, although citing the **Creaser** in a footnote (FN 6), the **Estabrook** reference says nothing of using Zn powder and instead uses a Pt electrode with the Co(III)sepulchrate as a mediator.

Thus the application of a the a Co(III)sepulchrate and metal powder in the use of biological systems would not at all be predictable in view of the cited art and in view of the state of the art.

Furthermore, looking at the state of the art in general, and not only at the cited references, Appellants respectfully assert that there is still no motivation for use of Zn powder for hydroxylation of fatty acids. Appellants wish to note the work of *Fang*, which also references the work of *Faulkner*, both of which were noted in Appellants IDS. *See Fang*, "Dithionite-Supported Hydroxylation of Palmitic Acid by Cytochrome P450BM-3" Drug Metabolism and Disposition Vol. 24, No. 11 (1996) pgs, 1282-1285; *Faulkner et al.* "Electrocatalytically driven ω-hydroxylation of fatty acids using cytochrome P450 4A1" Proc. Natl. Acad Sci. Vol. 92 (1995) pgs 7705-7709.

Starting from the electrode-based electron donor system of *Faulkner*, *Fang* suggests employing dithionite as a reducing agent for the P450 enzyme. Thus, one of ordinary skill in the art, starting from the electrode-based electron donor system of **Estabrook**, would have expected success with a reducing agent which is soluble in the reaction mixture, as for example dithinonite, rather than the metal-based system of *Creaser* and *Sargeson* (US 4,497,737; cited by Examiner) which according to *Sargeson* (See col. 4, lines 60-65) was regarded as a suitable redox system for inorganic and organic synthesis rather than biosynthesis. Thus, in view of the state of the art, one of ordinary skill in the art would find no motivation to use a metal in powder from as a source of electrons.

Appellants respectfully assert that the claimed invention also produces superior and unexpected results. Unexpected properties can be used to show that a claimed invention is not obvious over the cited references. *See MPEP 2145*. Appellants refer to data from experimental work submitted in the reply of February 12, 2004. Similar to the experiments performed by **Estabrook**, and summarized in Table 1 of the reference, the Appellants compared reaction rates for the BM-3 mutant F87-A under conditions similar to the cited art and also to the claimed invention. Artificial substrate 12-pNCA was used as the enzyme substrate to measure activity via an optical test. Enzyme activity was measured in separate reactions in either the presence of NADPH or electrolysis/Pt-electrode (Estabrook) and NADPH or Zn dust and Co(III)sepulchrate as electron sources. With the NADPH reactions set to 100%, the results showed that Zn dust and Co(III) sepulchrate reaction had a relative reaction almost twice as great as that of the **Estabrook** example. These data show that the electron donor system of the instant claims is more suitable for enzyme reactions as claimed because of the higher reaction rates. Such results cannot be expected from the disclosures of the cited references and the knowledge in the art at the time of filing.

The Examiner argues that the relative rates of the electron donor system for **Estabrook** is irrelevant because the rejection is based on the combined disclosures of **Estabrook** with **Creaser** and **Conrad**. However, Applicants respectfully submit that such unexpected results demonstrate that the present claims and their properties would not at all be predictable in view of the cited references. Such unexpected results and properties need not be recited in the claims as suggested by the Examiner but can be used to demonstrate non-obviousness and that no prima facie case of obviousness can be established. *See MPEP 2145*.

In view of the above, Applicants respectfully request the above mentioned rejection be withdrawn.

In order to facilitate the resolution of any issues or questions presented by this paper, the Examiner is invited to directly contact the undersigned by phone to further the discussion.

The undersigned representative requests any extension of time that may be deemed necessary to further the prosecution of this application.

The undersigned representative authorizes the Commissioner to charge any additional fees under 37 C.F.R. 1.16 or 1.17 that may be required, or credit any overpayment, to Deposit Account No. 14-1437.

Conclusion

Having addressed all issues set out in the Office action, Applicants respectfully submit that the claims are in condition for allowance and respectfully request that the claims be allowed.

Respectfully submitted,
NOVAK DRUCE & QUIGG, LLP

/Jason W. Bryan/
Jason W. Bryan
Reg. No. 51,505

Jason.Bryan@novakdruce.com
1000 Louisiana Ave
53rd floor
Houston, Texas 77002
T: 713-571-3400
F: 713-456-2836